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Effects of Sulfur Oxide Pollutants on Respiratory Function, Particle Deposition and Bronchial Clearance

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EFFECTS OF SULFUR OXIDE POLLUTANTS ON RESPIRATORY FUNCTION, PARTICLE DEPOSITION AND BRONCHIAL CLEARANCE

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16. ABSTRACT

The effects of sulfur oxide pollutants on respiratory function, particle deposition, and bronchail clearance were explored in series of three studies, two on donkeys and one on humans. In the first study, the effects of one-hour inhalation exposures to 0.3 - 0.6 ug H_2SO_4 and $(NH_4)_2SO_4$ aerosols in the donkey were studied in terms of alterations in pulmonary flow resistance and dynamic compliance, and changes in the regional deposition and tracheobronchial mucociliary clearance of an inert test aerosol. In the second study, the effect of chronic inhalation exposures to sulfuric acid mist upon mucociliary clearance from the lungs was studied, using the donkey as an analogue for man. Four animals were exposed one hour per day, five days per week, for six months; two to a concentration of 102 ug/m^3, the others to 106 ug/m^3. In the final study, the mucociliary particle clearance and respiratory mechanics of twelve healthy nonsmokers exposed to 1/2 um H_2SO_4 at 0 (control), 100, 300, and 1,000 ug/m^3 for one hour per day for four days were examined.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulartory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality documents on pollutants for which national amvient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This study was funded by EPA cintract 68-02-1726 and includes descriptions of experimental studies on animals which were ended on April 15, 1978 as well as the studies on human volunteers which continued through the end of January 1979.

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Director
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ABSTRACT

The effects of sulfur oxide pollutants on respiratory function, particle deposition, and bronchial clearance were explored in a series of three studies, two on donkeys and one on humans. In the first study, the effects of one-hour inhalation exposures to 0.3 - 0.6 μm H_2SO_4 and $(NH_4)_2SO_4$ aerosols in the donkey were studied in terms of alterations in pulmonary flow resistance and dynamic compliance, and changes in the regional deposition and tracheobronchial mucociliary clearance of an inert test aerosol. In the second study, the effect of chronic inhalation exposures to sulfuric acid mist upon mucociliary clearance from the lungs was studied, using the donkey as an analogue for man. Four animals were exposed one hour per day, five days per week, for six months; two to a concentration of 102 μg/m^3, the others to 106 μg/m^3. In the final study, the mucociliary particle clearance and respiratory mechanics of twelve healthy nonsmokers exposed to ½ μm H_2SO_4 at 0 (control), 100, 300, and 1,000 μg/m^3 for one hour per day for four days were examined.

Effects of Sulfur Oxide Pollutants on Respiratory Function,

Particle Deposition and Bronchial Clearance

1.0 INTRODUCTION

This final report summarizes the work performed under Contract 68-02-1726 through its termination at the end of January 1979. It includes descriptions of experimental studies on animals which were ended on April 15, 1978 as well as the studies on human volunteers which continued through the end of January 1979.

The results of the work performed under this contract has been presented and discussed in the three technical papers which are incorporated into this final report, as parts 3.0, 4.0, and 5.0. Sections 2.1, 2.2, and 2.3 provide extended summaries of the individual papers.

2.0 SUMMARIES OF SUBSTUDIES PERFORMED

The work under this contract was divided into three substudies: 1) Effects of short-term exposures to sulfuric acid and ammonium sulfate aerosols upon bronchial airway function in the donkey; 2) Effect of chronic inhalation of sulfuric acid mist upon mucociliary clearance from the lungs of donkeys; and 3) Effects of sulfuric acid aerosols upon mucociliary particle clearance and respiratory mechanics in healthy non-smoking humans.

2.0 Effects of Short-Term Exposures to Sulfuric Acid and Ammonium Sulfate upon Bronchial Airway Function in the Donkey

We conducted a series of tests to study the physiological response of the lungs of four donkeys to H_2SO_4 and $(NH_4)_2SO_4$. The response was characterized in terms of changes in pulmonary flow resistance (R_L), pulmonary compliance (C_L), regional deposition, and mucociliary tracheobronchial

clearance. The sulfate aerosols were in the 0.3-0.6 μm size range.

Measurements of R_L and C_L were performed, using the esophageal balloon technique, following one hour exposures to $(NH_4)_2SO_4$ at mass concentrations up to 3.0 mg/m^3. No significant change in R_L or C_L was observed due to these exposures. However, normal intra-animal variability was found to be quite large, and, depending upon the specific donkey, a 25-40% change in R_L and 10-20% for C_L would have to occur before any change could be considered significant.

Monodisperse, insoluble ferric oxide aerosols tagged with a γ-emitting isotope were used to monitor any changes in regional deposition and clearance induced by exposure to the sulfur oxides.

The relative tracheobronchial tree vs. alveolar distribution of ferric oxide was not altered by prior, one hour inhalations of up to 3.1 mg/m^3 $(NH_4)_2SO_4$ or up to 1.4 mg/m^3 H_2SO_4. The lack of measurable effect upon deposition supports the lack of measurable effect upon R_L, since both indices would be affected by alterations in bronchial calibre.

Mucociliary clearance of ferric oxide was not affected by one hour exposures to $(NH_4)_2SO_4$ at concentrations up to 1.6 mg/m^3. However, significant effects were observed following one hour exposures to H_2SO_4. The responses differed in the different donkeys. Two of the four animals exhibited a progressive change in the control rate of clearance during the course of the year-long series of ∿12 one hour exposures to H_2SO_4 exposures.

The other two animals exhibited a definite, short-term slowing of clearance following single exposures to submicron H_2SO_4, but had no changes in clearance in control tests. There was, however, considerable inter-individual variability in response at comparable exposure levels, with one donkey responding at concentrations >∿700μg/m^3 and the other responding as low as

194 $\mu g/m^3$. The short-term effect of H_2SO_4 upon clearance does not become apparent until ∿30 minutes following initiation of acid exposure.

3.0 Effects of Chronic Inhalation of Sulfuric Acid Mist upon Mucociliary Clearance from the Lungs of Donkeys

The chronic exposure study involved the inhalation exposure of four donkeys via nasal catheters to 100 $\mu g/m^3$ of submicrometer H_2SO_4 for one-hour per day, five days per week for six months.

The animals were studied in pairs, with the initial series of chronic exposure tests being done on Donkeys Ethel and Gus. They had both exhibited transient, but no persistent effects following single one-hour exposures. The second series was done on Donkeys Kevin and Leon, who had not previously been exposed to any inhaled irritants. Each individual animal had a characteristic clearance rate which was quite reproducible on repeated test runs in the pre-exposure control series.

In all four animals, clearance times became erratic within the first week of exposure to the sulfuric acid mist, with the rate becoming significantly different from control on many test days. However, the degree and the direction of change in rate differed to some extent in the different animals.

Among the four animals, the most dramatic response was shown by Donkeys Leon and Kevin; of these two, Donkey Leon demonstrated the most severe effect from the acid exposure.

Donkey Leon exhibited transient slowing and speeding of clearance during the first two months of acid exposure. However, these transient slowdowns became more severe, and beginning in the fifth month and continuing until the end of the exposure series, a sustained impairment of clearance occurred, and clearance remained abnormal throughout four months of follow-up

measurements.

Donkey Kevin initially showed some transient slowing, but appeared to have returned to within normal limits during the first month of exposure. However, beginning within the fourth month, clearance became erratic and exhibited a progressive sustained slowing until the end of the exposure series, with abnormal clearance persisting throughout the four month follow-up after the termination of exposure.

Donkey Gus exhibited transient clearance slowdowns during the first three months of acid exposure, but returned generally to within normal limits after this period. The magnitude of clearance slowdowns in the last three months was much less then during the initial three months. Occasional excursions were observed during the follow-up period, with most tests tending towards the lower limit of normal clearance times.

Donkey Ethel also exhibited some transient slowdowns; however, the general trend was towards an accelerated rate of clearance beginning at about the third month of acid exposure. Subsequently, she exhibited clearance which was faster than that of her pre-exposure average in all but one test performance during the follow-up period.

The sustained, progressive slowing of clearance observed in Donkeys Kevin and Leon, two initially healthy and previously unexposed animals, is a very significant observation, since any persistent alteration of normal mucociliary clearance can have important pathological implications.

4.0 Effects of Sulfuric Acid Mist on Mucociliary Particle Clearance and Respiratory Mechanics in Healthy Non-Smoking Humans

Twelve healthy nonsmokers inhaled $\frac{1}{2}\mu m(\sigma_g=1.9)$ H_2SO_4 at 0 (control), 100, 300 and 1,000$\mu g/m^3$ for one hour via nasal mask in random sequence on four

separate days. Respiratory mechanical function was assessed by body plethysmography, partial forced expiratory maneuver and nitrogen washout before and ½, 2 and 4 hours after the H_2SO_4 exposure. A ^{99m}Tc tagged Fe_2O_3 aerosol (7.5μm AMAD, σ_g= 1.1) was inhaled ∿10 min. before each H_2SO_4 exposure, with flow rate = 1.0 lps, tidal volume = 1 liter and breathing rate = 15/min. Lung retention of the deposited radioactivity was monitored by two 12.5 x 5 cm D.NaI scintillation detectors located within cylindrical collimators and placed externally over the right midlung field. A tracheal probe containing six rectangular (1 x 4 x 2.75cm) collimated scintillation detectors was used to determine the tracheal mucus transport rates (TMTR's) of boli of activity.

No consistent changes in respiratory mechanics were observed following H_2SO_4 exposure at any level, but mucociliary clearance was markedly altered. In individuals whose control run tracheobronchial clearance half-times ($TB_{½}$) were greater than the mean, there was an increased rate of bronchial clearance ($\Delta\overline{TB}_{½}$ = -56 min., $p \leq 0.01$) following exposure to 100μg/m^3. Following exposure to 1,000μg/m^3, clearance was slowed ($\Delta\overline{TB}_{½}$ = +60 min., $p \leq 0.05$). At 300μg/m^3 there was a wide range of response with increases in some and decreases in others. The TMTR's for this group increased significantly ($p \leq 0.09$) with increasing concentration. On the other hand, for the individuals with faster than average control run clearance rates, their $TB_{½}$'s were unchanged following exposure at all 3 concentrations, while their TMTR's decreased with increasing concentration ($p \leq 0.10$). Thus, brief exposures to submicron sulfuric acid at concentrations that may be encountered in both the ambient and occupational environments can affect mucociliary clearance, an important pulmonary defense mechanism.

EFFECT OF CHRONIC INHALATION OF SULFURIC ACID MIST UPON MUCOCILIARY CLEARANCE FROM THE LUNGS OF DONKEYS

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ABSTRACT

The effect of chronic inhalation exposures to sulfuric acid mist upon mucociliary clearance from the lungs was studied, using the donkey as an analogue for man. Four animals were exposed one hour per day, five days per week, for six months. The mean mass concentration of acid mist was 102 $\mu g/m^3$ for two animals, and 106 $\mu g/m^3$ for the other two. The mass median aerodynamic diameter was $\sim$ 0.5 μm. Clearance was monitored by serial, external *in vivo* measurements of the retention of an insoluble, radioactively tagged ferric oxide aerosol which was inhaled following exposure to the acid mist.

Bronchial clearance became erratic within the first week of exposure; rates were significantly different, usually slower, than control on many test days, although the degree of response varied among the four animals. Two animals exhibited a sustained impairment of clearance towards the end of the six-month exposure period and continued to have erratic clearance during a three-month follow-up period. No changes in the regional deposition of the ferric oxide occurred during the course of the study in any of the animals.

It is proposed that alterations in bronchial mucociliary clearance may be an early, if not the first, physiologic effect resulting from the inhalation of sulfuric acid mist, and this may be a factor in the pathogenesis of chronic bronchitis in populations exposed to the sulfur oxide-particulate-complex in the ambient air, which often includes sulfuric acid.

INTRODUCTION

Sulfate is a major component of the ambient aerosol in many urban and rural areas (Altshuller, 1973; Tanner, et al., 1977). It is largely derived via atmospheric chemical reactions from sulfur dioxide (SO_2), which is emitted by regionally distributed sources (Altshuller, 1973; Weiss, et al., 1977), primarily those which burn fossil fuel. This sulfate fraction consists largely of sulfuric acid (H_2SO_4) and its products of neutralization with atmospheric ammonia (Tanner, et al., 1977; Weiss, et al., 1977). However, the amount which is present as H_2SO_4 is neither well defined, nor constant temporally or spatially. The main mass of the sulfate is in the 0.2 to 0.6 μm (MMD) range (Altshuller, 1976) and may, therefore, remain suspended for a considerable period of time following formation.

Epidemiologic and experimental evidence suggests that particulate sulfate, a chemical class which, as normally measured in the atmosphere, includes H_2SO_4, is a more serious health hazard than its precursor SO_2 or than many other classes of common ambient air pollutants (Amdur, 1969; Rall, 1974). This is important, since decreases in SO_2 levels in the eastern and midwestern portions of the U.S., due to stricter source controls in urban areas, have not been accompanied by a proportionate decrease in sulfate concentration (Altshuller, 1976).

Most experimental studies of the pulmonary irritancy of inhaled H_2SO_4 mists have involved measurement of some form of flow resistance. These have generally been considered to provide sensitive indices of effect. However, a recent study in this laboratory (Schlesinger, et al., 1978) demonstrated a slowing of mucociliary transport along the bronchial tree of donkeys during a series of twelve weekly one-hour exposures to acid mist at concentrations within the range 100 to 1,000 $\mu g/m^3$. None of these exposures produced any measurable changes in pulmonary resistance or compliance, suggesting that alterations of mucociliary function may be an early,

if not the first, physiologic indicator of acute response to sulfuric acid inhalation.

Community exposures to a pollutant are generally continuous, albeit highly variable. Although there have been studies of chronic exposure to H_2SO_4 in relation to changes in respiratory mechanics (Lewis, et al., 1969; Alarie, et al., 1973), there are no data on the effects of such exposure upon mucociliary clearance. Thus, a study was undertaken to characterize the effect upon clearance of long-term, low-level exposure to H_2SO_4 mist, using the donkey as an analogue for man. The animals were exposed to a nominal H_2SO_4 concentration of 100 $\mu g/m^3$ [$\sim$ 0.5 μm mass median aerodynamic diameter (MMAD)] for one hour per day, five days per week, for six months. In the previous study (Schlesinger et al., 1978), single one-hour exposures at 100 $\mu g/m^3$ produced no measurable effect on bronchial mucociliary clearance. These effects were seen only at concentrations of 200 $\mu g/m^3$ and above.

METHODS

Experimental Animal

Four healthy, adult donkeys were used in this study. The rationale for using the donkey as an animal model for mucociliary clearance in man has been described (Schlesinger, et al., 1978) and is based upon consistent and comparable clearance changes in both species following exposure to cigarette smoke and after administration of cholinergic drugs (Albert, et al., 1969, 1973, 1975; Foster, et al., 1976; Berger, et al., 1978). The animals were unsedated throughout the entire course of this study. One pair of animals was exposed to acid mist within each ot two, six-month exposure periods.

Generation of H_2SO_4 Aerosol

The system used for the generation of H_2SO_4 mist has been previously described in detail (Schlesinger, et al., 1978). Basically, aerosols were produced by nebulization of H_2SO_4 solution, using a DeVilbiss #40 nebulizer. The nebulizer output is mixed with filtered and heated dilution air and passed through a cooling/mixing chamber to sampling and breathing ports. Output air was maintained

at 28°C, 45% R.H. and atmospheric pressure.

The aerosols were sampled for mass concentration and particle size analyses using a pre-calibrated, multicyclone sampler (Blachman and Lippmann, 1974) containing two cyclone-filter samplers in parallel. The sulfate contents of the cyclones and back-up filters within the sampler were determined using the ASTM turbidimetric technique (ASTM, 1975), with a calibrated Bausch and Lomb spectrophotometer (Schlesinger, et al., 1978).

Measurement of Tracheobronchial Mucociliary Clearance and Regional Particle Deposition

The pattern of mucociliary clearance was obtained by external, serial measurements of the retention of monodisperse ($\sigma_g < 1.1$), $\sim$ 5 μm (MMAD), insoluble ferric oxide (Fe_2O_3) aerosols, which were tagged with a non-leaching, γ-emitting radioisotope, ^{99m}Tc ($T_{\frac{1}{2}}$ = 6 hr). The aerosols were produced with a spinning disc generator (Lippmann and Albert, 1967).

The apparatus used for the retention measurements has been described previously (Schlesinger, et al., 1978). It consists of an array of three pairs of 12.7 cm (5 in.) diam. x 1.2 cm (0.5 in.) NaI(Tl-activated) scintillation detectors mounted on a saddle which is worn by the donkey. With this arrangement, measurement of radioactivity may be performed in three regions: a major portion of the lung, the lower trachea, and the upper trachea. Clearance from the lung region is observed as a progressive decrease in the measured level of radioactivity (corrected for natural decay) due to the proximal transport of deposited particles within the bronchial tree; subsequent clearance waves may then occur in the lower and ultimately the upper tracheal region (see Figure 2, upper panel).

Clearance from the bronchial tree was analyzed in terms of a characteristic index, the bronchial clearance half-time [$T_{(50\%)}$], i.e., the time required for completion of one-half of the total tracheobronchial clearance of the ferric

oxide (Albert, et al., 1974; Berger, et al., 1978). This parameter has been shown to be sensitive to clearance alterations produced by various drugs and irritants, including H_2SO_4 (Lippmann, et al., 1977; Schlesinger, et al., 1978).

The rate of mucociliary transport in the trachea was also determined, in terms of the time displacement of the activity waves in successive measurement positions, based upon the points of one half-maximum count rate of the ascending portion of each wave. The actual tracheal transport rate (TTR) was then obtained by dividing the distance between tracheal measurement positions by this time interval displacement (Lippmann, et al., 1977).

Although clearance per se is not affected by the size of the ferric oxide used as a monitor, observed clearance patterns are influenced by changes in its deposition; thus, an index of regional deposition was also measured. Clearance of the ferric oxide from the tracheobronchial tree is assumed to be essentially completed within 24 hours following exposure; residual chest radioactivity remaining at this time is assumed to represent that portion of the inhaled aerosol which deposited within the alveolar region (Albert, et al., 1968; Lippmann and Albert, 1969). Thus, the index of regional deposition was the percentage of the initial lung burden cleared by 24 hours [$C_{(24)}$].

Each donkey has been found to have a very reproducible pattern of regional deposition and clearance for the 5 μm ferric oxide aerosol (Albert, et al., 1968); any changes in this pattern following exposure to H_2SO_4 would indicate that this latter aerosol was responsible for the alterations.

The donkeys were housed in an unheated shed during the course of this study. Because of the effect of ambient pretest temperature upon mucociliary transport rates and fractional deposition in the tracheobronchial tree, values for $T_{(50\%)}$, $C_{(24)}$, and TTR were corrected to room temperature (20°C), using the correction factors determined in this laboratory by Bohning, et al. (1976).

Aerosol Inhalation

The ferric oxide and H_2SO_4 aerosols were inhaled ad lib, through 0.95 cm (i.d.) nasal catheters extending into the nasopharynx (Schlesinger, et al., 1978). The catheters passed through a flexible film bag which extended over the muzzle, and which was sealed around the muzzle with tape. Excess aerosol and exhaled particles were collected on a filter canister. Sulfuric acid exposures were for one hour; ferric oxide exposures lasted less than five minutes.

Experimental Protocol

A series of 15 control tests were performed on each animal, to determine baseline values for $T_{(50\%)}$, $C_{(24)}$ and TTR, prior to the start of the H_2SO_4 exposures.

To determine any effect upon $T_{(50\%)}$, $C_{(24)}$ or TTR due solely to breathing from the sulfuric acid generation unit, these parameters were measured following five sham exposures in which the nebulizer was filled with water, and the animal inhaled from the generation unit for one hour. These sham exposures had no effect ($p > 0.05$) upon any of the measurement indices.

Two consecutive six-month exposure series were performed. In each, two animals were exposed to $\sim$ 100 $\mu g/m^3$ H_2SO_4 for one hour per day, five days per week for six months; this resulted in a total of 122 acid exposures per animal. Each exposure was performed at the same time of day. The actual mean concentration and particle size for the H_2SO_4 aerosols used in each of the two exposure series are presented in Table 1.

Clearance tests were performed two or three times each week during the acid exposure series, resulting in a total of 64 clearance tests on each animal; the ferric oxide was administered within one minute of the end of acid exposure on these days. Following the completion of the acid exposure series, a number of clearance tests were performed during an additional three month period.

Two of the animals used in this study, namely Donkeys Ethel and Gus, had been used in a previous study involving twelve individual one-hour H_2SO_4 exposures (Schlesinger, et al., 1978). There was, however, no evidence for any persistence of detrimental effects from this earlier study. Control values for $T_{(50\%)}$, $C_{(24)}$ and TTR in the current series were not significantly different for these two animals from the controls of the previous study ($p > 0.05$). The other two animals, Donkeys Kevin and Leon, had not previously been exposed to any airborne irritants.

RESULTS

The regional deposition of the tagged ferric oxide test aerosol obtained in control tests and in tests performed during the six-month acid exposure series is summarized in Table 2. There was no significant difference (two-tailed t-test, $p > 0.05$) between the mean $C_{(24)}$ of the control tests and those performed during the months of acid exposure, nor in the variability (F-statistic, $p > 0.05$) of $C_{(24)}$ measured during the control and acid exposure series in any of the animals.

Since the regional deposition pattern was unaffected by the acid exposures, any changes in $T_{(50\%)}$ were due solely to alterations in mucociliary clearance function.

Table 3 presents the mean $T_{(50\%)}$ of the ferric oxide aerosol for control tests and for tests performed during the acid exposure series. Comparison of the coefficients of variation of $T_{(50\%)}$ shows that the variability of $T_{(50\%)}$ increased during the acid exposure series in all of the animals, by factors ranging from 4.3 to 9.5 times that of control tests. This increase in variability over control was significant in all of the animals ($p < 0.05$). Although in all cases the mean $T_{(50\%)}$ for the tests performed during the acid exposure series was also significantly different from control ($p < 0.05$), this does not

provide information concerning trends in $T_{(50\%)}$ over the six months of acid exposure.

In order to compare individual values for $T_{(50\%)}$ of the ferric oxide aerosol obtained during the course of the H_2SO_4 exposure series and to observe any trends due to the acid mist, a tolerance interval for $T_{(50\%)}$ was developed for each donkey, based upon the control series of tests performed prior to the acid exposures. Each interval was computed such that there is a probability of 0.95 that at least 99% of the distribution of normal $T_{(50\%)}$ is included between the upper and lower limits (Cox, 1970). Any value which lies above or below these limits is significantly different from control ($p < 0.05$). These limits are shown in Figure 1, with results of clearance tests performed during and after the acid exposure series.

In all of the animals, clearance times became erratic within the first week of exposure to the sulfuric acid mist, with the $T_{(50\%)}$ becoming significantly different from control on many test days. However, the degree and the direction of change in rate differed to some extent in the different animals and with time.

Of the four animals, the most dramatic response was shown by Donkeys Leon and Kevin; of these two, Donkey Leon demonstrated the most severe effect from the acid exposures.

Donkey Leon exhibited transient slowing and speeding of clearance during the first two months of acid exposure. However, the transient slowdowns became more severe during continued exposure. Beginning in the fifth month and continuing until the end of the exposure series, a sustained impairment of clearance occurred. Clearance remained quite erratic during the three-month follow-up period.

Figure 2 shows a clearance curve obtained on Donkey Leon during acid exposures, compared to a composite control. The $T_{(50\%)}$ for the former test was over 300 minutes. This extremely long half-time is due to essentially no clearance for

the first four hours following inhalation of the acid mist and ferric oxide. Considerable delays in the onset of clearance have been observed previously in donkeys undergoing chronic exposure to another irritant, cigarette smoke (Albert, et al., 1971, 1974).

Donkey Kevin initially showed some transient slowing, but appeared to have returned to within normal limits during the first month of exposure. However, beginning within the fourth month clearance became erratic, and exhibited a progressive sustained slowing until the end of the exposure series. During the three-month follow-up period, clearance rate remained generally abnormal.

Donkey Gus exhibited transient clearance slowdowns during the first three months of acid exposure, but returned generally to within normal limits after this period. The magnitude of clearance slowdowns in the last three months was much less than during the initial three months. Occasional excursions were observed during the follow-up period, but most tests tended towards the lower limit of his normal clearance half-time.

Donkey Ethel also exhibited some transient slowdowns; however, the general trend was towards an accelerated rate of clearance beginning at about the third month of acid exposure. Subsequently, she exhibited clearance which was faster than the pre-exposure controls in some of the tests performed during the follow-up period.

Table 4 presents the mean values for TTR of the ferric oxide in control tests and in tests performed during the acid exposure series. In the latter case, TTR could only be determined in tests with clearly discernable tracheal peaks. Since severe depression of bronchial clearance is usually associated with an absence of such peaks (see Figure 2), the test data represents values from only some of the clearance tests performed during the exposure period. No significant difference ($p > 0.05$) was found in the means, or variability, of TTR in the control series compared to tests showing tracheal peaks which were performed during the acid exposure

series. Thus, the alterations in bronchial clearance which were observed in this study did not result in corresponding changes in mucociliary transport rates within the trachea.

DISCUSSION

Mucociliary clearance is the most important mechanism by which inhaled and deposited particles, including toxic and infectious agents, are removed from the conductive airways of the lungs. Abnormal elimination of tracheobronchial mucus is associated with, and is perhaps a factor in, the pathogenesis of chronic obstructive lung disease, especially chronic bronchitis (Wanner, 1977). Thus, it is important to determine the response of the mucociliary system to challenges by common air pollutants, especially at concentrations which are realistic in terms of general community and occupational exposures.

This study has demonstrated significant alterations in mucociliary clearance from the bronchial tree of healthy donkeys during the course of a series of exposures to sulfuric acid mist administered for one hour per day, five days per week, for six months. The concentration used, $\sim$ 100 $\mu g/m^3$, is within the range of short term maximum levels found in urban air (Amdur, et al., 1978) and is 10% of the current threshold limit value for daily 8 hour exposures of workers.

Although all four animals showed a loss of stability of mucociliary clearance rates, the more significant observation is the apparent sustained slowing of clearance in two previously unexposed animals, Donkeys Kevin and Leon.

Differences in the degree of acid effect between the four animals indicate a wide variability in sensitivity. This is not surprising. Most species, including man, do show large interindividual variability in response to inhaled irritants when various parameters are analyzed, including mucociliary clearance (Fairchild, et al., 1975; Schlesinger, et al., 1978).

Of the four animals, Donkeys Ethel and Gus appeared to be less affected by the acid exposures. Donkey Ethel does show a trend towards a speeding up of clearance during and after the acid exposure series. On the other hand, clearance in Donkey Gus returned to essentially normal levels within the first three months of exposure. Recovery of mucociliary function during chronic irritant exposure has been observed previously. In a study of chronic exposure of donkeys to cigarette smoke (Albert, et al., 1971), in which all three animals studied showed an initial period of clearance slowdown, one of them exhibited a decrease in the level of impairment during further exposures. Since Donkeys Ethel and Gus had been exposed to sulfuric acid mist in a previous study (Schlesinger, et al., 1978), it is possible that they adapted more readily to the exposures in the current study. On the other hand, in this earlier study, these donkeys did not show the gradual alteration in their baseline rates of clearance which were exhibited by the two other animals undergoing a similar protocol. This appears to indicate that Donkeys Ethel and Gus represent the least "susceptible" individuals in the total population of six animals exposed to sulfuric acid mist in the current and previous study.

Neither a slowing nor speeding of mucociliary clearance, if sustained, is beneficial; both are, most likely, indications of a pathophysiological response of the bronchial tree to the acid mist. Furthermore, slowing of clearance may result in the prolonged retention of deposited particles, increasing the risk of local damage or systemic uptake of noxious materials.

Mucus transport depends upon the effective transfer of mechanical energy from the cilia into linear motion of an overlying (gel) layer of mucus. Alterations in transport rate could result from: 1) changes in ciliary beat rate, 2) changes in the thickness and/or viscosity of the gel and/or sol components of the mucus blanket, 3) changes in the level of secretions from mucus-secreting elements in the bronchial tree, or 4) some combination of these factors. For

example, accelerated transport may occur if beat rate is increased of if changes in the viscosity of the gel layer results in a more efficient energy transfer from the cilia. Some degree of increase in secretions would tend to result in an acceleration of mucus transport, while too much of an increase could result in a sol layer thicker than the height of the cilia, and a reduction in rate. Unfortunately, there are no data allowing determination of the relative roles each factor plays in the net responses observed in this study.

There are very few data concerning the effects of long-term exposure to low levels of H_2SO_4; those which exist are not directly related to clearance changes. Alarie, et al., (1973) exposed cynomolgus monkeys to various concentrations of H_2SO_4, essentially continuously, for 78 weeks, and examined the lung for histological changes. The lowest concentration used was 480 $\mu g/m^3$ (0.54 μm MMD), and no effects were observed at this level. Guinea pigs were also exposed to H_2SO_4, at 100 or 80 $\mu g/m^3$ (2.8 and 0.8 μm) for 52 weeks; no alterations in pulmonary histology which could be related to acid exposures were noted in those animals either. A chronic exposure series which did provide some indirect evidence for possible alterations of clearance was that of Thomas, et al. (1958), although concentrations used were much higher than in the present study. Guinea pigs which were continuously exposed to H_2SO_4 for periods from a few weeks to over five months at mean concentrations ranging from 2.6 to 4.1 mg/m^3 (0.9 μm), and 1.5 to 2.7 mg/m^3 (0.6 μm), showed a decrease in the amount of mucus in what the authors called "major and minor bronchi."

Previous studies which directly examined mucous clearance generally involved single acid exposures, at fairly high concentrations. Fairchild, et al. (1975), exposed mice for four hours to H_2SO_4 mist at 15 mg/m^3 (3.2 μm CMD) and found retarded clearance from the lungs, with no morphologic evidence of tissue damage. Newhouse, et al. (1978) found that two-hour exposures during exercise to 1 mg/m^3

(0.58 μm MMD) resulted in a speeding-up of bronchial clearance from the lungs of men. Schlesinger, et al. (1978), demonstrated transient slowing of bronchial clearance in donkeys following single exposures to submicron acid aerosols having concentrations as low as ∿ 200 $\mu g/m^3$. Sackner, et al. (1978) exposed sheep for 20 minutes to submicron aerosols of H_2SO_4 up to 14 mg/m^3 and for 4 hours to 4 mg/m^3 and found no change in tracheal mucous velocity. However, the current study and that of Schlesinger, et al. (1978) have demonstrated that severe changes in bronchial clearance were not associated with any alteration in tracheal transport rate. This situation was also observed in the study of Albert, et al. (1974), who found that bronchial clearance was altered in donkeys exposed to the whole fresh smoke from two cigarettes, while tracheal transport was unchanged.

The reason for the lack of effect upon tracheal transport is unclear; perhaps the trachea is more resistant to irritant effects, or the small size of the irritant aerosols in these studies precluded significant deposition within this airway.

Based upon the current and previous studies, it is suggested that alterations in bronchial mucociliary clearance may be a very sensitive response of the lungs to inhaled irritants, occurring before there is evidence from more conventional physiological tests of irritant response. It may, in fact, be a first indication of potential lung damage. Changes in clearance may begin soon after chronic irritant exposure (within one week in this study) and, perhaps, continue for an uncertain amount of time at a subclinical level, prior to development of any manifest symptoms of obstructive lung disease. For example, Goodman, et al. (1977) examined tracheal transport rates in chronic bronchitis. They observed that some young smokers demonstrated various degrees of impairment of tracheal transport, yet had no overt symptoms and had normal pulmonary function, including tests of airway obstruction.

Epidemiological evidence suggests a relation between sulfur oxide pollution

and chronic bronchitis (Reid, 1964; Ellison and Waller, 1978), but factors other than air pollution, such as cigarette smoke, tend to complicate the matter. Changes in respiratory mechanics in the general population appear to be associated with exposure to sulfur oxides (Lawther, et al., 1973, 1974a, 1974b), although the most clearcut alterations occur at high levels, i.e., >500 $\mu g/m^3$. In any case, it is questionable whether this type of response can explain these epidemiological suggestions. It is more likely that interference with mucociliary clearance may result in pathological effects which are of greater consequence than alterations of mechanics per se. Furthermore, the exact agent, or agents, within the mixed sulfur oxide atmosphere which is primarily responsible for irritant potency is not known; sulfuric acid may be an important factor in this regard. Amdur and Underhill (1968) suggest that a large part of the irritant effect of SO_2 may actually be due to conversion to H_2SO_4, since the combination of various aerosols with SO_2 potentiated irritant effects of the latter in the guinea pig; the aerosols presumably aided in the oxidation of the gas to sulfate.

The most direct evidence for an association between chronic bronchitis and exposure to sulfuric acid comes from occupational exposures; but these are at much higher levels than used in the current study, or than found in the ambient air. Williams (1970) observed an excess incidence of chronic bronchitis in workers occupationally exposed to H_2SO_4 levels above 1 mg/m^3 (probable mass median diameter = 1.4 µm); however, the excess was actually an increased incidence of episodes in afflicted workers, rather than an increase in the number of workers afflicted. This is consistent with findings that acute air pollution episodes primarily affect people with already compromised lung functions. However, the effects upon the mucociliary system observed in the current study suggest that sulfuric acid may initiate disease, as well as exacerbate it. This is supported by two other studies. In two previously healthy human subjects, Sim and Pattle (1957) found the development of what appeared to be long lasting symptoms of

bronchitis due to repeated exposures to H_2SO_4 lasting one hour and given no more than twice a week with at least 24 hours between exposures. Concentrations were high, ranging from 3-39 mg/m^3. In a study of weekly exposures of donkeys Schlesinger, et al. (1978) noted that during the series and for some time after, two of four previously healthy animals demonstrated a change in their baseline clearance rate, towards a more persistent slowing. Finally, in the current study, two of the donkeys continued to show clearance abnormalities for at least three months after the acid exposures ceased.

Exposure to cigarette smoke, an agent known to be involved in the etiology of chronic bronchitis in man, produces effects upon mucociliary clearance in the donkey which are quite similar to those produced by sulfuric acid. The inhalation of whole fresh smoke from ten or more cigarettes resulted in marked transient slowing of bronchial clearance (Albert, et al., 1974) comparable to those seen following one-hour exposures to H_2SO_4 (Schlesinger, et al., 1978). Six month chronic exposure to cigarette smoke (Albert, et al., 1971) resulted in comparable effects upon clearance to those observed in the current study, including, depending upon the specific donkey, the development of progressively more abnormal rates initially, some recovery towards normal, and occasional accelerated clearance during continued exposure.

Interspecies differences in sensitivity to exposure to irritants make extrapolating from animal exposures to the human situation uncertain. However, based upon demonstrated response similarities of the mucociliary system in donkeys and man using cigarette smoke, this study suggests that daily exposure to fairly low levels of sulfuric acid may produce alterations in bronchial mucociliary transport as an early sign of pulmonary dysfunction in previously healthy individuals. In addition to implications concerning the general population, these results are also of significance in terms of occupational exposures, since the current eight-hour TLV for H_2SO_4 is 1000 µg/m^3, which permits cumulative daily exposures 80 times higher than those used in this study.

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TABLE 1

Characteristics of H_2SO_4 Aerosol

	Mass Concentration ($\mu g/m^3$)		Particle Size (MMAD)[a] (μm)	
	Mean	Standard Deviation	Mean	Standard Deviation
Exposure Series I (Donkeys Ethel and Gus)	102	35	0.46	0.17
Exposure Series II (Donkeys Leon and Kevin)	106	40	0.51	0.16

[a]The geometric standard deviation (σ_g) of the H_2SO_4 aerosol size was ~1.5.

TABLE 2

Regional Deposition of Ferric Oxide Test Aerosol

Donkey	Mean Tracheobronchial Deposition (% of initial lung burden cleared by 24-hours, $C_{(24)}$)	
	Control	Acid Exposure Series
Ethel	69 (6)[a]	71 (7)
Gus	70 (6)	74 (7)
Kevin	55 (8)	56 (7)
Leon	58 (5)	56 (6)

[a]Standard Deviation

TABLE 3

Bronchial Clearance of Ferric Oxide Test Aerosol

Donkey	Control		Acid Exposure Series		Variability Increase Factor[a]
	Mean $T_{(50\%)}$ (min)	Coefficient of Variation (%)	Mean $T_{(50\%)}$ (min)	Coefficient of Variation (%)	
Ethel	21 (3)[b]	14.3	29 (18)[b]	62.1	4.3
Gus	24 (3)	12.5	31 (27)	87.1	7.0
Kevin	23 (3)	13.0	40 (30)	75.0	5.8
Leon	31 (3)	9.7	85 (78)	91.8	9.5

(a) Represents the coefficient of variation for clearance tests performed during the six months of acid exposure divided by the coefficient of variation of control tests.

(b) Standard deviation

TABLE 4

Tracheal Transport Rate of Ferric Oxide Test Aerosol

Donkey	Mean Tracheal Transport Rate (cm/min)	
	Control	Acid Exposure Series
Ethel	0.9 (0.1)[a]	0.8 (0.1)[a]
Gus	1.0 (0.2)	1.0 (0.3)
Kevin	0.6 (0.4)	0.7 (0.4)
Leon	0.8 (0.2)	0.9 (0.2)

(a) Standard deviation

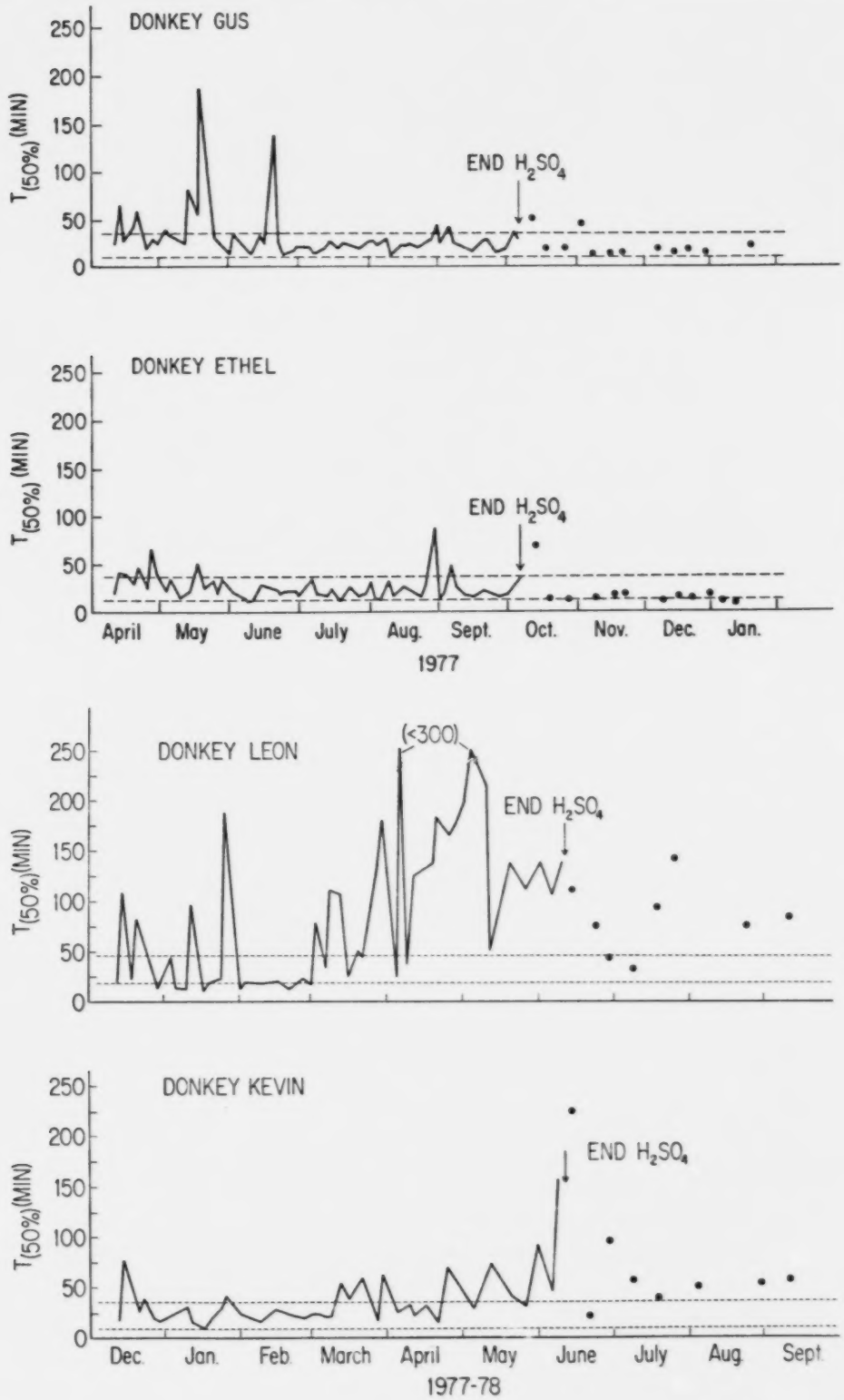

Figure 1 Bronchial clearance half-times during the course of the six-month series of daily one-hour exposures to H_2SO_4 mist, and during the three-month follow-up period. The dotted horizontal lines delineate the tolerance interval for each animal.

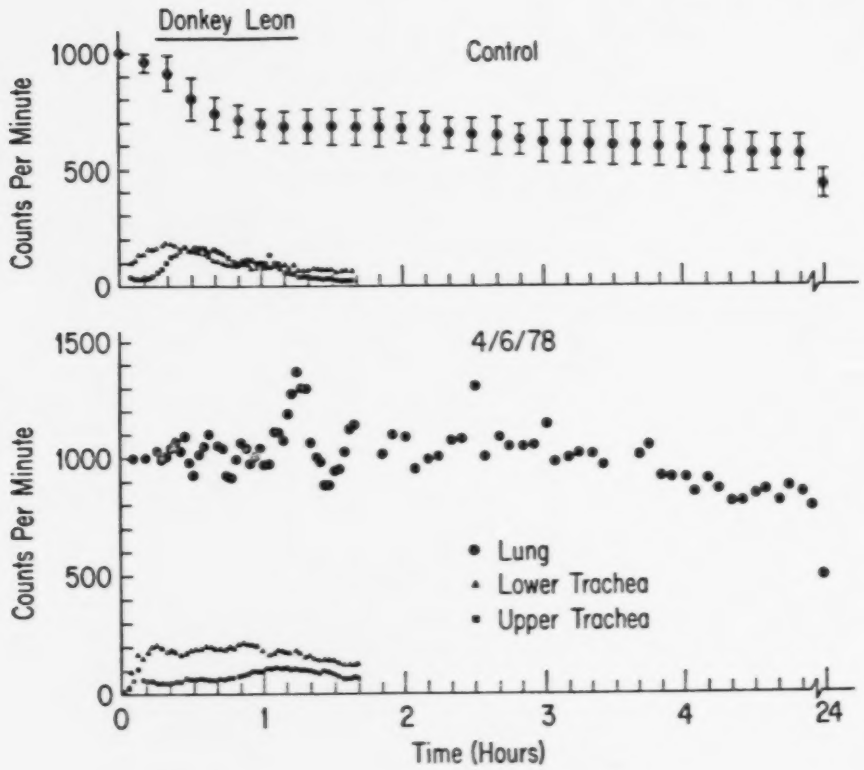

Figure 2 Clearance curves of radioactively tagged ferric oxide test aerosol for Donkey Leon. Count rates are normalized to 1000 per minute in the lung position. The upper panel shows a composite control curve; each point in the lung position represents the mean of 15 tests and the lines through each point represent ± 1 standard deviation. The lower panel shows the clearance curve obtained following the exposure to H_2SO_4 on 4/6/78. Note the lack of lung clearance during the first four hours after ferric oxide inhalation and the lack of discernable tracheal position peaks.

Sulfuric Acid Aerosols: Effects on Mucociliary Particle Clearance and Respiratory Mechanics

Abstract Twelve healthy nonsmokers inhaled ½μm(σ_g=1.9) H_2SO_4 at 0 (control), 100, 300 and 1,000μg/m^3 for one hour via nasal mask in random sequence on four separate days. No consistent changes in respiratory mechanics were observed following H_2SO_4 exposure at any level, but mucociliary clearance was markedly altered at all three concentrations either in terms of bronchial clearance half-times or tracheal transport velocities.

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The health consequences of environmental exposures to airborne sulfur oxides are complex and poorly understood. The sulfur oxide compounds in the ambient air are a complex mixture of various chemical species in both gaseous and particulate phases. Until recently, toxicoligical and legislative concerns for the adverse health effects associated with these compounds were focussed on the irritant effects of sulfur dioxide gas (SO_2). In controlled clinical experiments, SO_2 was shown to be a mild pulmonary irritant capable of producing reversible bronchostriction, but only at concentrations that would have to be considered unrealistic when compared to the ambient experience (1). There is increasing recognition that coexposures to SO_2 and various particulates produce synergistic effects, and that transformations of SO_2 into more oxidized particulate compounds leads to enhanced potency. This has led to increased concern for, and research on, the physiological effects of inhaled sulfates.

A major constituent of the ambient sulfate aerosol is sulfuric acid (H_2SO_4). Together with its ammonia neutralization products, H_2SO_4 forms the dominant mass fraction of the accumulation mode (0.1 - 2.5 μm) of the ambient aerosol (2). Sulfuric acid is distributed over a large portion of the Eastern United States and Northern Europe, and ambient concentrations often range from 10 to 20 $\mu g/m^3$. It arises from primary sources such as automobiles equipped with catalytic converters and secondary sources such as chemical conversion of gaseous SO_2 to particulate forms during long-range transport of ambient air masses. The major reductions in recent years of ground level SO_2 concentrations in the East and Midwest have not been accompanied by proportional reductions in sulfate aerosol concentrations (2).

In order to study the possible effects that transient H_2SO_4 exposures might have on human pulmonary physiology, we exposed 12 healthy nonsmoking volunteersto 0.5µm (MMD), (σ_g = 1.9)H_2SO_4 aerosol in concentrations of 0 (distilled water control), 113 ± 33, 311 ± 18, and 993 ± 139 µg/m^3 ($\bar{X}$ ± SD) (3,4). The lowest level is representative of an acute air pollution episode which might occur once or twice a year in densely populated regions of the United States. The highest level is comparable to the current 8-hour concentration limit of 1.0 mg/m^3 for chronic occupational exposure (5).

Sulfuric acid aerosol undergoes several chemical and physical transformations as it passes throughthe respiratory tract. Larson et al. (6) found significant amounts of ammonia (NH_3) gas in the expired air of humans. The oral airways contain more NH_3 than the nasal airways, presumably due to bacterial decomposition of salivary urea. Ammonia levels measured at the mouth (29 to 520 µg/m^3 NH_3) could stoichiometrically convert up to 1500 µg/m^3 H_2SO_4 to $(NH_4)_2SO_4$, while the lower levels measured in air exhaled through the nose (13 to 46 µg/m^3NH_3) could convert up to 130 µg/m^3H_2SO_4. Consequently, nasal breathing was selected for the exposures in this study in order to provide a realistic simulation of normal human exposure conditions and to minimize the in-situ neutralization of the H_2SO_4 to the less irritating $(NH_4)_2SO_4$.

Since sulfuric acid is hygroscopic, ambient aerosol droplets exposed to the warm humid air of the respiratory tract will grow in size and decrease in acid molarity. The aerosol employed in this study was delivered in an atmosphere at 25.6 ± 1.8°C and 46 ± 5% relative humidity. Within the warm,

saturated air of the upper respiratory tract, this aerosol is expected to double in diameter (7). The physiological result would be an enhancement of bronchial airway deposition. Since chronic obstructive pulmonary disease (COPD) is essentially a disease of the small airways (8), where the protective, mucus layer is quite thin and, perhaps, discontinuous (9), alterations in particle size which affect deposition efficiency have toxicological implications. The deposition patterns of particles within the airways will be very different from that of a soluble gas such as SO_2, which is almost completely absorbed in the nasopharynx (10).

In order to investigate the possible effects of H_2SO_4 on the bronchomotor tone of both the large and small airways, we measured three parameters of ventilatory mechanics. Airways resistance to airflow (R_{aw}) was determined by body plethysmography (11); maximal expiratory flow at 25 percent vital capacity (V_{25}) was determined by the partial-forced exhalation technique (12); and the distribution of ventilation was determined by the multiple breath nitrogen washout technique (13). This battery of tests took about 30 minutes to perform and was conducted before and within 10 minutes after the one hour H_2SO_4 exposure. These tests were repeated twice more at approximately 2 and 4 hours after cessation of acid exposure.

In addition to conventional measurements of ventilatory function, we also monitored mucociliary particle clearance by two methods. Mucociliary clearance is the dominant defense mechanism of the tracheobroncial tree. Particles and gases in the tidal air may be deposited on, or absorbed into, the mucous layer covering the conducting airways. Once captured by

this protective barrier, materials are transported out of the lungs as the mucus is moved by the underlying cilia of the columnar epithelium.

One of the two approaches used to monitor mucociliary transport involved the measurement of the thoracic retention of a monodispersed ferric oxide (Fe_2O_3) aerosol tagged with γ-emitting technetium-99m. This aerosol was deposited on the bronchial mucus during a 2 minute inhalation period which was ∿10 min before the H_2SO_4 exposure. Reproducible Fe_2O_3 aerosol deposition was insured by controlling the particle size (7.5μm AMAD, $\sigma_g \leq 1.1$), inspiratory flow rate (1.0 ℓps), tidal volume (1.0 ℓ), and breathing rate (15/min). Serial retention of the deposited radioactivity was monitored by two (12.7 x 5.1 cm) NaI(Tl) scintillation detectors mounted within cylindrical collimators. The paired detectors were aligned externally over the right midlung field (14). The second method employed was the measurement of the rate of mucociliary transport in the trachea (15). The large particle size of the radioactive test aerosol and high inspiratory flow provided sufficient large airway deposition for measurement of boluses of activity by an external tracheal probe consisting of six rectangular (1 x 4 x 2.75 cm) collimated NaI(Tl) scintillation detectors. Measurements were made while subjects sat in a low background chamber with both detection systems in place. Our noninvasive techniques allowed the measurement of tracheal transport velocity to be made simultaneously with measurements of right lung bronchial retention. The time in which boluses could have been detected was during a fixed 90 minute period which started about 2 hrs after the inhalation of the Fe_2O_3 aerosol and 40 min. after the end of the

inhalation of the H_2SO_4. Boluses were not observed for 2 of 12 persons after all exposures. The failure to observe boluses in these tests could have been because clearance was essentially complete prior to the tracheal transport measurements, or because the amount of activity was below the level of detection.

Mucociliary clearance was monitored throughout the day of the 1-hour acid exposure, by which time at least 90 percent of the tagged aerosol is cleared in most individuals. Each subject returned the following day in order to determine the 24-hour retention. Previous studies have shown that mucociliary clearance is essentially complete within this time with the fraction of material remaining having initially been deposited in nonciliated pulmonary spaces (e.g., alveoli and respiratory bronchioles) (14).

The sulfuric acid exposures in these experiments appear to have had little, if any, effect on ventilatory mechanics. Of the pulmonary functions tested, only slight increases (3.1 and 4.6%) in the average measured airway resistance were observed immediately after exposures to 100 and 300 $\mu g/m^3$, respectively. Following 1000 $\mu g/m^3$, however, there was no significant change in airway resistance. The other pulmonary function tests were not significantly altered following any exposure. Our results are consistent with those of recent studies conducted elsewhere in both animals and humans, and indicates that ambient levels of sulfuric acid are not likely to cause significant alterations in respiratory mechanical function following short-term exposures (16). However, we cannot rule out the possibility of effects from chronic low-level exposures.

In contrast to the absence of major effects on respiratory mechanical function, mucociliary clearance was markedly altered in most of the individuals following exposure at all levels. The responses for the ten persons in which both types of mucociliary transport data were obtained are summarized in Figure 1. We divided our subjects into two distinct groups based on their control test clearance half-times. In persons with slower than average clearance (i.e. persons with tracheobronchial clearance half-times ($TB_{\frac{1}{2}}$) greater than that of the mean of all twelve subjects), there was an increase in bronchial clearance rate ($\overline{\Delta TB_{\frac{1}{2}}}$ =-56 min, $p \leq 0.01$) upon exposure to 100 $\mu g/m^3$ H_2SO_4. Following exposure to 1000 $\mu g/m^3$, however, clearance became significantly slower ($\overline{\Delta TB_{\frac{1}{2}}}$ = +60 min, $p \leq 0.05$). At 300 $\mu g/m^3$ the response was varied, with some persons showing faster clearance and some persons showing slower clearance in comparison to their control tests.

In the lungs of the other group, i.e., those with faster than average clearance in the control test, the $TB_{\frac{1}{2}}$ appeared to be unaffected by exposure to H_2SO_4. One possible explanation for the absence of observed effects might be that clearance was essentially complete before a sufficient dose of H_2SO_4 was administered. In previous animal studies in this laboratory, effects were first noted only after 30 minutes of exposure to H_2SO_4 at similar concentrations (17). In this group the mean $TB_{\frac{1}{2}}$ was 43 minutes, which includes ~10 minutes between the inhalation of Fe_2O_3 and H_2SO_4, and ~30 minutes of H_2SO_4 exposure. Another possible explanation may be that deposition of 7.5 μm Fe_2O_3 test aerosol was, in this group, more concentrated within the proximal airways, and that these airways

were relatively unaffected by the much smaller H_2SO_4 aerosols.

Inherent to the measurements of respiratory function used in this study, is the greater contribution due to large rather than small airways. For example, airway resistance is dominated by an upper airway component, so that even if the acid exposure had produced a major change which was confined to the smaller airways (18) it could have gone undetected. The wide variability of the other respiratory function tests employed also limit our conclusions, but if there was a significant change in the sizes of the proximal airways, it would have been detected.

Figure 1 also shows that there was a correlation (r^2=0.81) between the control values of $TB_{\frac{1}{2}}$ and tracheal mucociliary transport rates (TMTR's), i.e. persons with faster than average bronchial clearance also have faster than average TMTR's. However, following exposure to H_2SO_4 at concentrations as low as 100 $\mu g/m^3$, this relationship breaks down (r^2=0.11).

If the distal airways were the main loci of H_2SO_4 deposition and, consequently, the region most likely to respond, then one would not expect to see a large change in the tracheal transport. The data shown in Figure 1 are consistent with this hypothesis. In persons with slower than average bronchial clearance, TMTR's increased with increasing acid concentration. In persons with faster than average bronchial clearance, however, there were some dose-related decreases in TMTR's. Since the observation period in which the tracheal detector was in place was fixed, a possible explanation is that boluses recorded for each group came from different regions. In persons with slower than average clearance the observed TMTR's may have been determined from material that was initially deposited in airways closer to the trachea, i.e. predominately proximal airway deposition. In this group the TMTR would therefore reflect boluses from an area in which the effect

induced by the H_2SO_4 was less severe. In contrast, in those persons with faster than average clearance, the material making up the boluses may have been deposited more distally, in an area more affected by the acid exposure. Therefore, the initial boluses could be faster than the subsequent boluses.

Clearly, our evidence indicates H_2SO_4 aerosol affects the mucociliary defense mechanism of the distal airway of the lung. In order to function properly, mucociliary clearance is dependent upon both the physicochemical properties of the mucus and the coordinated beating of the underlying cilia. Various *in vitro* techniques isolating each parameter may be useful in exploring mechanisms by which H_2SO_4 can cause mucociliary dysfunction. Mucus is excreted into the airway lumen in an alkaline form which is then acidified by carbon dioxide (19). *In vitro* studies have shown that in high pH solutions mucus is soluble while at lower pH it becomes viscous, perhaps accounting for the sol-gel bilayer of mucus that lines the airways (20). Therefore, the hydrogen ion supplied by the deposited H_2SO_4 aerosol may stiffen the mucus lining and increase the efficiency of removal. This is consistent with the initial increase in bronchial clearance we observed in humans following exposure to 100 $\mu g/m^3$. Additional *in vitro* studies have shown that in vivo exposures at $\sim$1,000 $\mu g/m^3$ may cause depression of ciliary beating frequency (21) and, with coexposure to 0.5 ppm of ozone, increase in mucus secretion rates (22), both of which may lead to a depression in overall bronchial clearance. Since bronchial clearance is dependent upon the pre-existing state of the mucous layer, increases or decreases in clearance in healthy persons cannot be considered beneficial, especially when considering persons with chronic respiratory diseases in which this system has already been disturbed.

The dose-response trend observed in this experiment parallels previous studies conducted in this laboratory with cigarette smoke, one known causal factor in the complex etiology of chronic bronchitis. Bronchial clearance is markedly accelerated when healthy persons smoked several cigarettes (23). However, the fresh smoke from 10 or more cigarettes produce substantial retardation of bronchial clearance (24). Recent animal studies in this laboratory (25) have also shown that repeated exposures to H_2SO_4 at 100 $\mu g/m^3$ are able to produce a marked and persistent depression in bronchial clearance comparable to that produced by chronic cigarette smoking (24), even for those animals which demonstrated no transient effect following exposures at 100 $\mu g/m^3$ (17).

Unfortunately, direct evidence for a causal link between chronic low level exposures to sulfuric acid and chronic bronchitis is lacking. One epidemiological survey of persons occupationally exposed to H_2SO_4 at concentrations which probably far exceeded those used in this experiment found an increase in the number of attacks of respiratory diseases, particularly bronchitis, though the number of persons with respiratory diseases was not increased (26). Persistent bronchitic symptoms have also been reported in a clinical study following repeated exposures to SO_2 and H_2SO_4 (27), but again the exposure levels were very much greater than those used in this study. Based on the results of this study, and in consideration of the results of the recent coordinate animal studies (17,25), it appears that chronic H_2SO_4 exposures at the levels used in this experiment could produce persistent changes in mucociliary clearance in previously healthy individuals, and may lead to the exacerbation of pre-existing respiratory disease.

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3. The health status of each volunteer was determined by a clinical examination including chest X-ray, blood and urine analysis, and pulmonary function tests. Nonsmokers were selected because of the varied effects that smoking has on mucociliary clearance, either due to acute exposure, long-term exposure, or abstention from chronic use.
4. Sulfuric acid concentrations were determined in two ways: During exposure direct measurements were made with a Meloy Model 285 Flame Photometer Detector. An integrated sample was also collected on a glass-fiber filter and analyzed by the barium chloride-turbimetric ASTM technique.
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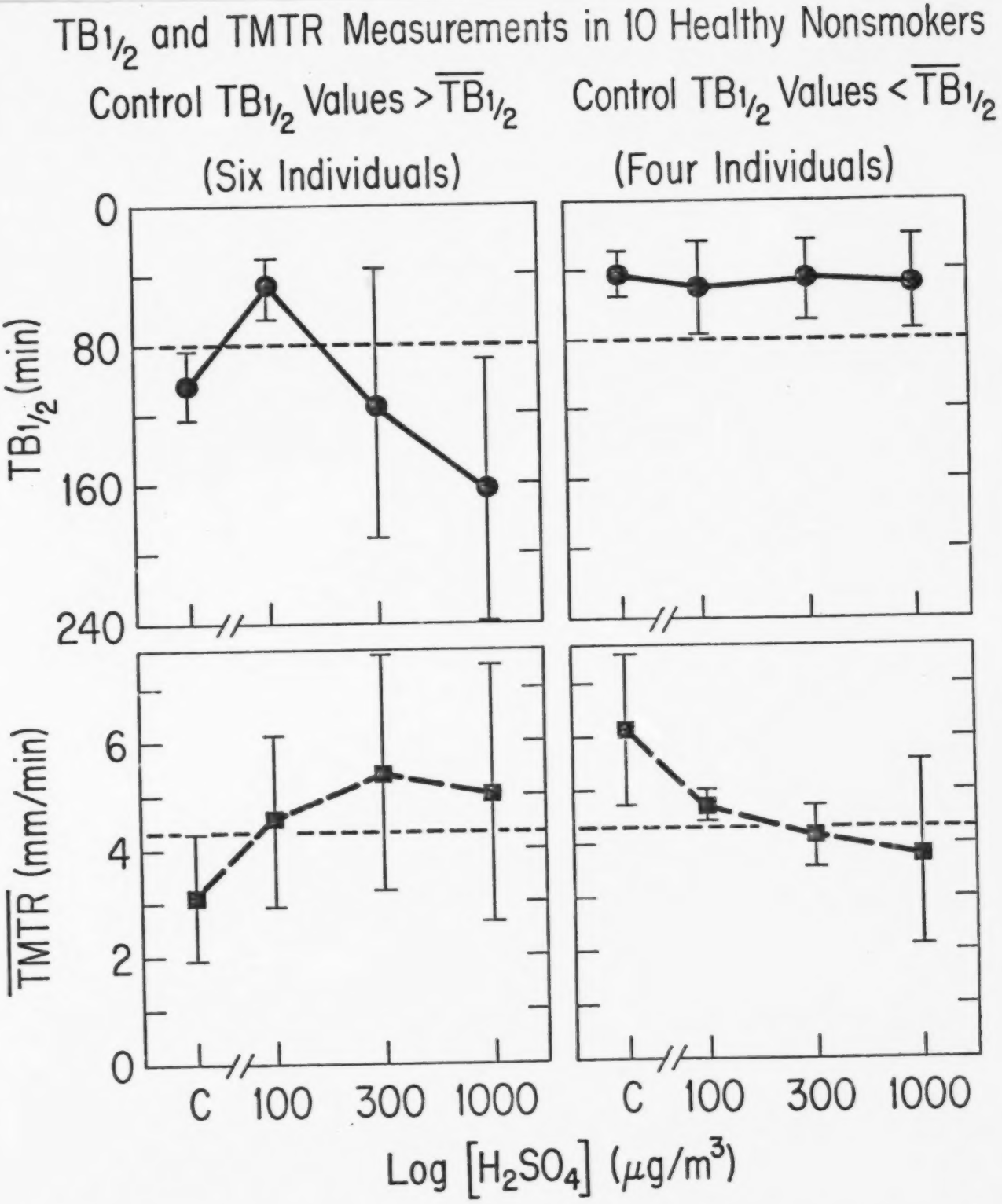

Figure 1. Effects of one-hour exposures via nasal mask to ½µm sulfuric acid aerosols at concentrations of 0 (control), 100, 300 and 1,000µg/m^3 on tracheobronchial clearance half-time ($TB_{\frac{1}{2}}$) and tracheal mucus transport rate (TMTR) of ^{99m}Tc-tagged Fe_2O_3 aerosol inhaled immediately preceding the H_2SO_4 exposure.